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Relative and absolute density estimates of land planarians (Platyhelminthes, Tricladida) in urban rainforest patches

Fernando Carbayo[1,2,3]
Odair Matarensi Junior[1]
Marília Jucá[1]

ABSTRACT

Land planarians (Platyhelminthes) are likely important components of the soil cryptofauna, although relevant aspects of their ecology such as their density remain largely unstudied. We investigated absolute and relative densities of flatworms in three patches of secondary Brazilian Atlantic rainforest in an urban environment. Two methods of sampling were carried out, one consisting of 90 hours of active search in delimited plots covering 6,000 m² over a year, and the other consisting of leaf litter extraction from a 60 m² soil area, totaling 480-600 l leaf litter. We found 288 specimens of 16 species belonging to the genera Geobia, Geoplana, Issoca, Luteostriata, Obama, Paraba, Pasipha, Rhynchodemus, Xerapoa, *and the exotic species* Bipalium kewense *and* Dolichoplana striata. *Specimens up to 10 mm long were mostly sampled only with the leaf litter extraction method. Absolute densities, calculated from data obtained with leaf litter extraction, ranged between 1.25 and 2.10 individuals m^{-2}. These values are 30 to 161 times higher than relative densities, calculated from data obtained by active search. Since most common sampling method used in land planarian studies on species composition and faunal inventories is active search for a few hours in a locality, our results suggest that small species might be overlooked. It remains to be tested whether similar densities of this cryptofauna are also found in primary forests.*

Key-Words: Geoplaninae; Berlese funnel; Soil fauna; Cryptofauna; Richness; Manual sampling.

INTRODUCTION

Some taxonomic groups of soil inhabitants, such as land planarians (Platyhelminthes, Tricladida, Geoplanidae), are rarely mentioned in studies of biodiversity. This may be a reflection of their paucity in the northern hemisphere, where most studies have been conducted (*see,* Platnick, 1991), also due to their cryptic habits (Dendy, 1890) and insignificant ecological role, as Jones & Boag (1996) suggested for the native British land flatworms.

Contrarily, in subtropical Central-South Brazilian biotopes land planarians likely constitute a significant component of the soil fauna (Froehlich, C.G.,

[1] Universidade de São Paulo – USP, Escola de Artes, Ciências e Humanidades, Laboratório de Ecologia e Evolução. Rua Arlindo Bettio, nº 1.000, Vila Guaraciaba, CEP 03828-000, São Paulo, SP, Brasil.
[2] Universidade de São Paulo – USP, Instituto de Biociências, Programa de Pós-Graduação em Zoologia, Departamento de Zoologia. Rua do Matão, Travessa 14, nº 321, Butantã, CEP 05508-090, São Paulo, SP, Brasil.
[3] Email of corresponding author: baz@usp.br
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1966). In these biotopes and those from South Brazil, species richness can be very high (*e.g.,* Carbayo & Leal-Zanchet, 2000; Leal-Zanchet *et al.,* 2011) and even reaches the world's highest numbers (Sluys, 1999), although only a small proportion of their species is known (Froehlich, E.M. & Carbayo, 2011). Dendy (1890) coined the term cryptofauna for assemblages of a variety of light-abhorring animals, like land flatworms, arthropods and mollusks that, during daylight, usually live under logs and stones and under the rotten bark of trees, where they find adequate humidity. Since land planarians have no mechanism for resisting desiccation (Kawaguti, 1932), they strongly depend on the humidity of its microhabitats to survive (Schirch, 1929; Froehlich, C.G., 1955; Winsor *et al.,* 1998). Nocturnal habits, negative phototaxis, and deep burrowing in dry conditions are adaptations of the land planarians to dribble the lack of water-saving mechanisms (Kawaguti, 1932; Ogren, 1955; Froehlich, C.G., 1955; Sugiura, 2009).

Land flatworms are carnivores, mostly predators (Ogren, 1995; Winsor *et al.,* 2004). In the last decades, the high detrimental ecological and economical impact of a few invasive land flatworms has triggered a number of works on their distribution and density. In New Zealand, Christensen & Mather (1998) found high densities (up to 16 flatworms m^{-2}) of the so-called "New Zealand flatworm" *Arthurdendyus triangulatus* (Dendy, 1894) under pine logs in grasslands bordering *Nothofagus* forest and grasslands (forest clearance). However, in potato fields in the Faroe Islands (Denmark), where the species was accidentally introduced, densities can be as high as 110 specimens m^{-2}. This species is considered a pest due to its ability to nearly eliminate earthworm populations from grass fields (Blackshaw, 1990), mostly affecting species with low rates of reproduction (Jones *et al.,* 2001).

In the Brazilian virgin forests, the moistened refuges are varied and thus, finding flatworms is usually complex (Schirch, 1929). In dry days, however, finding them becomes easier in open sunlit areas like clearings in the woods, grasslands and roadsides, since they congregate in humid pockets such as under stones and rotten logs (Schirch, 1929; Froehlich, C.G., 1955). Flatworms have been found displaying random and aggregate distributions (Antunes *et al.,* 2012; Murchie *et al.,* 2003). Aggregate distribution in refuges certainly causes bias in density estimates (Murchie *et al.,* 2003). In the USA, Ogren (1955) found a maximum abundance of *Rhynchodemus sylvaticus* (Leidy, 1851) of 2-8 individuals per square foot of wood boards placed on the soil as traps. Blackshaw (1990) calculated densities of *A. triangulatus* in an Irish soil grassland by using two methods, traps and formalin expellant in the soil, and demonstrated the unreliability of traps as a means of estimating population density but sampling with formalin expellant provided to be a good density method (Blackshaw, 1990). As for native flatworm fauna, as far as we know, there is only one study addressing density estimates; by active search of worms on the soil during the day in a natural Brazilian Araucaria forest, Antunes *et al.* (2012) calculated a relative density of 0.0066 individuals m^{-2} of a flatworm assemblage.

In most taxonomic studies, samplings in the field are conducted by direct, visual search on the soil litter. However, due to their cryptic habits, an unknown fraction of these organisms is likely overlooked.

In this work, we aimed to estimate the ratio of the dwelling land planarians that are collected by active search in three secondary Brazilian Atlantic rainforests. We calculated relative densities of these organisms and then compared them with absolute densities estimated from samplings with a Berlese funnel-inspired device.

MATERIAL AND METHODS

We selected three forest patches in São Paulo city (Brazil): Previdência Park (herein called Previdência, located on -23.580608, -46.727048) with ~ 9 ha; Butantan Forest (Butantan, -23.566329, -46.720242) with ~ 2 ha; and Horto Osvaldo Cruz (Horto, -23.567197, -46.716827) with ~ 2 ha. The three patches are at ~ 760 m above sea level and the climate is humid subtropical (*Cfa* categorization, Peel *et al.,* 2007), with hot humid summers and mild dry winters. The patches, nestled in a strongly urbanized territory, are covered with secondary Atlantic rainforest. Horto and Butantan are ~ 0.2 km distant from each other and ~ 1 km from Previdência. The history of these forest patches is unclear. At the beginning of the 20th century, Butantan was mainly a pasture area and a *Eucaliptus* forest on gentle hills (Hoehne, 1925, p. 40). By 1918, it was transformed into a garden of medicinal herbs, and then to plantations. After a period, the forest recovered the area and since 2000 it has been closed to public visitation (Yamada, 1995). Before 1917, Horto was a plantation area, including manioc, sugar cane and grasses (Hoehne, 1925; p. 40). In 1970, it was used for experimental studies with insects and mammals, and in 1992 trails were opened for guided visitation (Oliveira *et al.,* 2005). Previdência is covered with the native plants *Tibouchina granulosa* (Melastomataceae), *Cariniana estrellensis* (Raddi) Kuntze (Lecythidaceae), *Syagrus* *romanzoffiana* (Cham.) Glassman (Arecaceae), *Cecropia pachystachya* Trec. (Cecropiaceae), and also exotic species such as chinaberry tree (*Melia azedarach*) (Meliaceae) and *Delonix regia* (Bojer ex Hook.) Raf. (Caesalpiniacae). Previdência has suffered from some fires and nowadays there are trails available for public visitation (Querido, 1999; see also Candiani *et al.,* 2005).

From March 2007 to May 2008 we visited 10 times the three patches, each visit approximately every 45 days. We estimated relative and absolute densities of flatworms by means of two sampling methods. One method consisted of active visual search for animals under leaf litter and fallen tree branches between 10 h and 13 h (performed by OMJ and MJ) in order to estimate relative densities of flatworms. This is the method conventionally used by most researchers carrying out faunistic studies of flatworms. In each of the three patches we delimited four plots of 1 × 50 m; one collector searched for flatworms in two plots, dedicating 45 minutes per plot. Another collector searched for flatworms in the remaining two plots also for 45 minutes. In each visit, all three patches were sampled. In subsequently visits we delimited four new plots in another place of the same patches and conducted new searches following the same process. At the end of the 10 visits, each collector had searched for flatworms over 1,000 m^2 during 15 h. Data gathered by the two collectors were treated together. The flatworms were placed in 50 ml plastic vials and brought to the laboratory for identification and storage in 80% ethanol.

On the same day of each visit, one of us (MJ) followed the second sampling method, herein called as leaf litter extraction. We used this method for calculating absolute densities of flatworms. With a small gardening shovel we removed nearly all leaf litter (excepting logs of more than 60 cm in length or 10 cm in diameter) from two plots in each patch, each plot measuring 1 m^2 and 8-10 l of volume. We put the leaf litter collected from each plot in individual plastic bags, brought them to the laboratory, and maintained under natural light at room temperature. The same sampling day, logs were manually broken into minor fragments and each the leaf litter of each bag was put in a Berlese funnel-inspired device in the day of sampling. The device consists of a basin fastened on the top of another one, both measuring 60 cm diameter and 20 cm high. The top basin had in its base 120 holes of 1 cm in diameter. In the lower basin we placed an irregularly folded white cotton cloth moistened with mineral water. A 100 W incandescent lamp under a conical lampshade, was installed 30 cm above the top basin, to illuminate and heat the leaf litter placed in the upper basin. This technique stimulated the organisms to move down through the holes into the folds of the moistened cloth. Every three days we removed the uppermost and dried leaf litter layer, detached the top basin and collected the specimens that had moved down to the bottom basin. Animals found were collected and the basin refastened. Leaf litter dried completely after periods ot time varying between two and five weeks depending on the rain water contained in the leaf litter at the sampling moment.

We calculated the success of sampling by active search as the rate of mean number of specimens m^{-2} found by active search divided by the mean number of specimens m^{-2} found with the leaf litter extraction method.

Specimens were identified by examining the external morphology. When needed, also the internal morphology was studied by means of histological sections (anterior end, pharynx and copulatory apparatus) stained with the Mallory-Heidenhain method as modified by Cason (1950). Undescribed species and immature individuals having similar body shape and color pattern were referred as Geoplaninae.

RESULTS AND DISCUSSION

We collected a total of 288 individuals, 182 by active search and 106 using leaf litter extraction (Table 1). In Horto were found 38% of the individuals, in Previdência 38% and in Butantan 24%. Some individuals died and decomposed during transportation or in the laboratory. A total of 16 species were identified. Additionally, two morphospecies were found, namely Geoplaninae 1 and Geoplaninae 2; these two are represented by small-sized (less than 10 mm in length after fixation), immature individuals so they could not be identified. Interestingly, no individual of these morphospecies was recorded with active search; it is seemingly due to the poor capacity of human eye to find such small organisms within the forest. Since most common sampling method used in studies on species composition and faunal inventories of these organisms is the active search for a few hours in a locality, small species might be overlooked.

Relative densities, measured from data yielded by active search (54 sec/m^2) were very low: 0.013 individuals m^{-2} (Butantan), 0.042 individuals m^{-2} (Horto), and 0.035 individuals m^{-2} (Previdência). Nevertheless these estimates are ~ 2-6 times higher than 0.0066 individuals m^{-2} calculated by Antunes *et al.* (2012) in Southern Brazil, even though they sampled for 1 min 36 sec m^{-2}. This difference might be related to the state of conservation of the forests,

TABLE 1: Abundances and density of land planarians found in three forest patches; Horto, Butantan and Previdência, in São Paulo city (Brazil). Specimens were collected by active search on the soil and by leaf litter extraction with a Berlese-funnel inspired device.

Sampled Area	**Horto**		**Butantan**		**Previdência**		
Sampling Method	**Active**	**Device**	**Active**	**Device**	**Active**	**Device**	**Both**
Geoplana quagga Marcus, 1951	47	10	5	8	35	16	121
Issoca rezendei (Schirch, 1929)	4	3	—	—	26	6	39
Obama burmeisteri (Schultze & Müller, 1857)	22	—	—	—	1	—	23
Geoplaninae 1	—	1	—	12	—	4	17
Geoplaninae 2	—	3	—	12	—	2	17
Xerapoa pseudorhynchodemus (Riester, 1938)	—	1	—	1	4	9	15
Pasipha cf. *astraea* (Marcus, 1951)	1	—	13	—	—	—	14
Bipalium kewense Moseley, 1878	4	2	—	2	—	—	8
Dolichoplana striata Moseley, 1877	2	3	1	1	—	—	7
Geoplaninae 3	—	—	5	2	—		7
Luteostriata ernesti (Leal-Zanchet & E.M. Froehlich, 2006)	4	1	—	—	—	—	5
Pasipha pasipha (Marcus, 1951)	1	—	—	2	1	—	4
Rhynchodemus sp. 1	—	—	—	1	1	1	3
Geoplaninae 4	—	—	2	—	—	—	2
Paraba multicolor (von Graff, 1899)	—	—	—	—	2	—	2
Geoplaninae 5	—	1		1		—	2
Geobia subterranea Schultze & Müller, 1857	—	—	—	—	—	1	1
Geoplana cf. *vaginuloides* (Darwin, 1844)	—	—	—	—	1	—	1
Individuals	85	25	26	42	71	39	288
Relative (in parentheses) and absolute densities, in individuals m^{-2}	(0.042)	1.25	(0.013)	2.10	(0.035)	1.95	

peculiar ecological properties of the communities, or even difficulties in seeing in the darker shading resulted from the dense canopy of the *Araucaria* forest.

Absolute densities obtained were 1.25 (Horto), 1.95 (Previdência), and 2.10 (Butantan) individuals m^{-2}, or 30-to-161 times higher than relative densities (30× in Horto, 161× in Butantan, 56× in Previdência). After 30 hours of active search in each patch over a year, we collected 0.62-to-3.36% of extant soil-dwelling flatworms (0.62% in Butantan; 1.79% in Previdencia; 3.36% in Horto) as calculated from the ratio of relative: absolute density. We did not further studied the causes of differences in density values; they might be related to specific histories undergone by each of the forest patches but their histories are very poorly known.

Considering the low fraction of individuals collected over a year, concerns about depletion of flatworm fauna caused by collection might be disregarded. Furthermore, density estimates from leaf litter extraction are certainly underestimated because (a) animals dwelling deep into soil column, inhabiting large logs or even specimens which may have climbed on the vegetation were not taken with the leaf litter method; and (b) some animals might also have died in the elapsed time between sampling and leaf litter dry-out due to their sensitivity to moisture and heat (Winsor, 1997). Thus, the number of animals collected must only be a small fraction of the actual population.

As far as we know, this is the first study showing absolute density estimates for mostly native flatworm species in secondary forests. Estimates of leaf litter collect method herein showed are much higher than the apparent density calculated from active search. The leaf litter collect method is a promissory and an alternative sampling technique that could help to understand the ecological role that this neglected component of cryptofauna plays in nature. We suggest the use of this technique to study flatworm densities in primary forests.

RESUMO

As planárias terrestres (Platyhelminthes) são importantes componentes da criptofauna do solo, mas aspectos relevantes da sua ecologia, tais como riqueza e densidade, ainda são amplamente desconhecidos. Investigamos aqui as densidades relativa e absoluta das planárias terrestres de três manchas de mata atlântica secundária. Adotamos dois métodos de amostragens. Num método dedicamos 90 horas durante um ano à busca ativa de planárias em um conjunto de áreas demarcadas que somaram 6,000 m². O segundo método consistiu na extração das planárias de 480-600 l de serapilheira retirados de um total de 60 m²

de solo. Encontramos 288 espécimes pertencentes a 16 espécies de planárias dos gêneros Geobia, Geoplana, Issoca, Luteostriata, Obama, Paraba, Pasipha, Rhynchodemus, Xerapoa, *além das espécies exóticas* Bipalium kewense *and* Dolichoplana striata. *Os espécimes com até 10 mm de comprimento foram coletados principalmente por extração. As densidades absolutas, calculadas a partir dos números obtidos da extração, oscilaram entre 1,25 e 2,10 indivíduos* m^{-2}. *Estes valores são de 30 a 161 vezes maiores que as densidades relativas, calculadas a partir da busca direta. Como, na maioria dos estudos sobre composição de espécies e inventários faunísticos o tempo dedicado às coletas dura umas poucas horas por localidade, nossos resultados sugerem que espécies pequenas podem passar despercebidas. Ainda está por determinar-se se valores semelhantes de densidade desta criptofauna são também observados em bosques primários.*

Palavras-Chave: Geoplaninae; Funil de Berlese; Criptofauna; Fauna do solo; Riqueza; Coleta manual.

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